Localization of the Novel Serine/Threonine Protein Phosphatase 6 Gene (*PPP6C*) to Human Chromosome Xq22.3

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Serine/threonine protein phosphatases, which regulate many cellular processes including cell cycle regulation, can be differentiated by their action on *in vitro* substrates and by their sensitivity to the potent inhibitor and strong tumor promoter okadaic acid into four classes: PP1, PP2A, PP2B, and PP2C (3). In addition to isoforms of these enzymes, cDNA cloning has revealed an increasing number of novel serine/threonine protein phosphatases. Recently, we identified protein phosphatase 6 (PP6), a novel catalytic subunit from human cells that shows a sequence identity of 56% to human PP2A. We demonstrated that PP6 represents a functional homologue of the cell cycle regulatory serine/threonine phosphatases Sit4p from *Saccharomyces cerevisiae* and ppe 1 from *Schizosaccharomyces pombe*, which are highly similar to human PP6, with amino acid identities of 61 and 68%, respectively (1). Here, we report the chromosomal localization of the PP6 gene (*PPP6C*) by fluorescence *in situ* hybridization, followed by fine-mapping on a YAC contig.

We used a cloned 1230-bp cDNA of PP6 based on our published sequence (EMBL sequence database, Accession No. X92972) to screen a YAC library spotted onto two nylon membranes (a gift of Hans Lehrach, Berlin). Hybridization was performed as described (2), and of 4×10^4 YACs two clones containing the *PPP6C* gene were identified (ICRFγ901N086Q and IRCFy900E1138Q). After interspersed repetitive sequence long-range PCR (6), DNA from both YACs was labeled with biotin-16–dUTP (Boehringer, Mannheim) by nick-translation and hybridized *in situ* to methanol/acetic acid-fixed human metaphase chromosomes (5). Hybridization sites were detected by FITC-conjugated avidin, and chromosomes were counterstained by DAPI (4-6-diamino-2-phenylindole-dihydrochloride). A cooled CCD camera (Photometrix, Tucson, AZ) was used to generate digitized images for both fluorochromes, which were overlaid electronically after digital processing. Both YACs containing *PPP6C* showed a single hybridization signal to Xq22–q23 (Fig. 1).

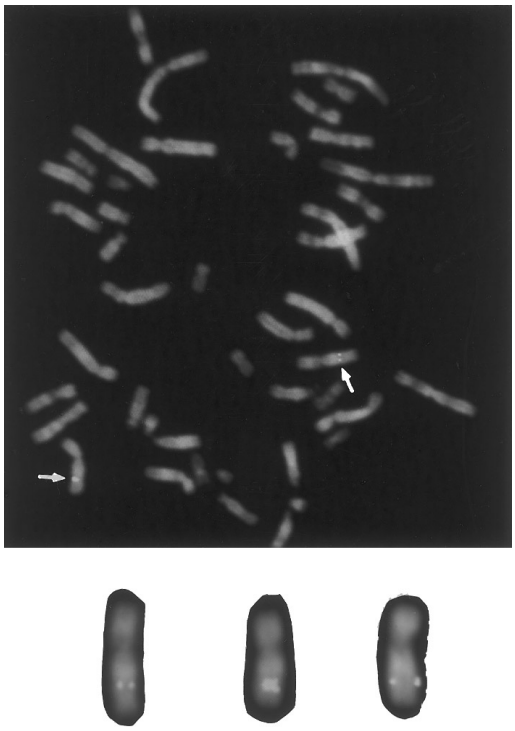

FIG. 1. Fluorescence *in situ* hybridization of human metaphase spread. Hybridization using YACs ICRFy901N086Q and ICRFy900E1138Q containing the *PPP6C* gene results in a single signal on chromosome Xq22–q23.

To refine the localization of *PPP6C* on the long arm of chromosome X further and to verify the results obtained by FISH, we mapped the gene on a YAC contig spanning the entire Xq21.33–q22.3 region (E. Kendall, manuscript in preparation), using a 1230-bp PP6 cDNA. The PP6 cDNA was radiolabeled and hybridized to filters containing DNA from all the YAC clones from Xq22.1–q22.3. *PPP6C* was found to lie on YAC clones 74G9 (ICRF), 145A9 (ICRF), 149C6 (ICRF), 28AD10 (ICI), 31FB6 (ICI), 810B1 (CEPH), 744A12 (CEPH), 823F2 (CEPH), and 940G5 (CEPH) and to be localized between markers DXS1210 and DXS456 in Xq22.3. Both markers are distal to the *COL4A5* gene and about 1–2 Mb proximal to the marker DXS287, which lies near the Xq23 border (E. Kendall, manuscript in preparation).

Serine/threonine protein phosphatases of type 1 and 2A are the cellular targets of the potent tumor promoter okadaic acid, suggesting that members of this class of enzymes are potential tumor suppressors (4). The chromosomal localization of *PPP6C* to chromosome Xq22.3 is of interest, because PP6 as a cell cycle regulatory protein phosphatase could represent a potential tumor suppressor. To date, however, no tumor suppressor loci have been mapped to this chromosomal region.

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The Gene Encoding the Schwann Cell Protein Periaxin Localizes on Mouse Chromosome 7 (*Prx*)

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The *Prx* gene encodes the periaxin protein, which is specifically expressed by myelin-forming Schwann cells in the mammalian peripheral nervous system (PNS) (5). Although periaxin is initially concentrated in the adaxonal membrane (apposing the axon), as myelin sheaths mature periaxin becomes predominately localized at the abaxonal Schwann cell membrane (apposing the basal lamina) (8). We have proposed that the shift in localization of the protein in the Schwann cell after completion of the spiralization phase of myelination reflects the participation of periaxin in the membrane–protein interactions that are required to stabilize the mature sheath. Defects in this protein may underlie certain human peripheral neuropathies since several forms of Charcot–Marie–Tooth disease Type I, a peripheral demyelinating condition, have been shown to be caused by mutations in genes that are expressed in myelinating Schwann cells (1, 6, 9). This new understanding of the molecular genetics of CMT disease has intensified the search for novel genes involved in PNS development.

Here we report the chromosomal localization of *Prx* using the European Collaborative Interspecific Backcross (EUCIB) facility. The murine *Prx* gene was first localized by Southern blotting (7) of a ^{32}P-labeled probe (4) that comprised nucleotides 1–2992 of the rat periaxin cDNA (5) to *Eco*RV-digested F2 DNAs from progeny of *Mus spretus* and C57BL/6 crosses that were provided by the EUCIB. *M. spretus* and C57BL/6 yielded *Eco*RV fragments of 14 and 17 kb, respectively. The segregation pattern of the RFLPs in 44 mice indicated that the fragments were located on mouse Chromosome 7 and mapped between the anchor markers *Pkcc* and *D7Nds5*. To gain a more accurate localization we amplified a 70-bp fragment and a 65-bp fragment of the periaxin gene in *Mus musculus* and *M. spretus*, respectively, using a forward primer sequence at the 3′ end of exon 4 and a reverse primer within the fourth intron (Ex4F, ATGGAGGCCAGGAGCCGCAGCGCTGA; In4R, CCTACGTCCTCTACAGTGAC). The segregation of *Prx* with *Pkcc* and *D7Nds5* was analyzed, and the results are shown in Fig. 1. The anonymous marker *D7Mit77* cosegregated with *Prx* in 14 animals typed.

The assignment of the periaxin gene to mouse Chromosome 7 was confirmed by fluorescence *in situ* hybridization (data not shown). Metaphase preparations were made from an exponentially growing culture of mouse embryonic stem cells, which were incubated for 1 h in 0.1 μg/ml Colcemid before harvesting. The cells were treated with hypotonic 0.5% trisodium citrate, 0.25% KCl for 10 min at 37°C before fixation

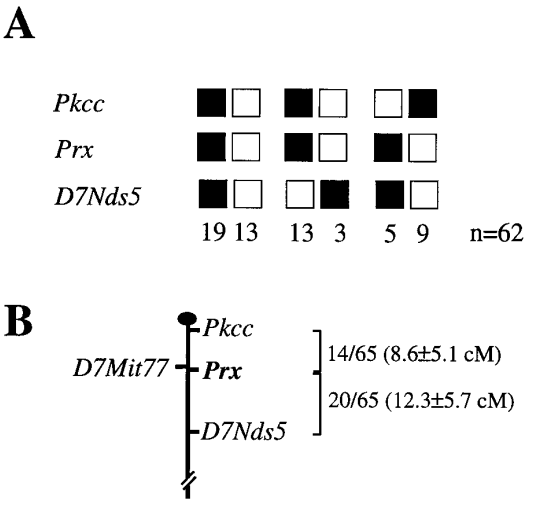

FIG. 1. Chromosomal location of *Prx*. (**A**) Haplotype analysis of 62 mice from the EUCIB interspecific backcross for the markers *Pkcc*, *Prx*, and *D7Nds5*. The solid boxes represent mice scored for both *Mus musculus* and *Mus spretus* alleles, and the open boxes represent mice scored for either *spretus* or *musculus* but not both. The number of offspring inheriting each type of chromosome is indicated at the foot of each column. The marker *D7Mit77* is not included due to the low number of mice typed. (**B**) A partial genetic map of Chromosome 7 that shows distances in centimorgans including standard errors between *Prx* and the two anchor markers *Pkcc* and *D7Nds5*. The cosegregating marker *D7Mit77* is shown on the left. Linkage distances are not drawn to scale.

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